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First record of *Hyphopichia burtonii* isolated from the storage pest *Sitophilus zeamais* and its bioactivity against mycotoxigenic fungi

M.N.P. Seco[1,2], A.K.M. Beltran[1] and M.A.O. Balendres[1,3,4]*

Summary Corn weevil (*Sitophilus zeamais)* is one of the most destructive pests of corn seeds during storage. The weevil may be a vector of mycotoxigenic fungi or yeast contaminating seed lots. In this study, an unknown yeast species was isolated from corn weevils found in stored corn seeds. We hypothesized that this yeast had an antifungal activity thereby inhibiting growth of mycotoxigenic fungi in corn seeds. The yeast species was identified as *Hyphopichia burtonii*, using combined morphological and molecular assays, and its potential inhibitory activity was assessed *in vitro* (spread plate and dual culture) against three known mycotoxigenic fungi, *Fusarium verticillioides, Aspergillus niger* and *A. flavus*. Screening of the antagonistic activity of the yeast isolate showed 50 – 69% colony growth inhibition of three fungi when the yeast was spread plated on PDA but only slight inhibition (5.8 – 13.7% growth inhibition) in the dual culture assay. The sporulation of the fungi was also affected at 57 – 96% and 29 – 40% in spread plating and dual culture assay, respectively. In addition, volatile and non-volatile fractions also showed a reduction in mycelial growth. Variable responses were observed among the mycotoxigenic fungi. Further research would be interesting on the potential utilization of the antagonistic yeast to reduce fungal growth and sporulation, and possible mitigation of mycotoxin contamination in corn grains. To our knowledge, this is the first record of *H. burtonii* isolated from an insect, specifically *S. zeamais*.

Additional keywords: corn weevil, insect-yeast interaction, seed pathology, yeast

Introduction

Corn (*Zea mays*) is one of the major staple crops grown throughout the Philippines, amounting to 2 million metric tons (Philippine Statistics Authority, 2021). About 14 million Filipinos prefer white corn as their main staple while yellow corn accounts for about 50% of livestock mixed feeds (Department of Agriculture). Much of it is produced by small-farm holders for their consumption and livelihood. Corn grits are used for human consumption, while the other parts of the seeds produced during milling may be used as animal feeds. Corn seeds are often stored prior to processing, particularly during the rainy season. Some of these seeds are also used for the next growing season.

During the postharvest and storage period, seeds are predisposed to several fungal contaminants and insect pests, which may contribute to economic loss and reduce seed quality (Balendres *et al.,* 2019). These biotic agents are estimated to cause 20% of food losses, of which up to 40-50% are from developing countries (Yun *et al*., 2018). Among the stored product pathogens and pests are *Aspergillus* species, *Fusarium* species, and *Sitophilus zeamais* (Coleoptera: Curculionidae) (corn weevil) (Ferreira-Castro *et al*., 2012).

Mycotoxins like aflatoxin, ochratoxin, and fumonisin are toxic substances produced by some fungi (mycotoxigenic fungi), namely *Aspergillus flavus, A. niger,* and *Fusarium verticillioides* (Balendres *et al*., 2019). The

[1] Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines, Los Baños, Laguna, Philippines.
[2] Department of Science and Technology-Science Education Institute, Philippines.
[3] Department of Biology, College of Science, De La Salle University, Manila, Philippines.
[4] Plant and Soil Health Research Unit, Center for Natural Sciences and Environmental Research, De La Salle University, Manila, 1004 Philippines.
* Corresponding author: mark.angelo.balendres@dlsu.edu.ph

Food and Agriculture Organization's (FAO) approximates 25% of the world's annual crop production to be contaminated with mycotoxins, leading to significant crop losses in food and feed products valued at an estimated 1 billion metric tons (Pfliegler *et al*., 2015). Meanwhile, infestation of insects in mature cobs during grain storage is common. The corn weevil, *S. zeamais,* is one of the most serious and common pests of corn in the tropics (Paes *et al*., 2012; Sori and Ayana, 2012). It has also been reported as a vector for toxigenic fungi during storage (Ferreira-Castro *et al*., 2012); thus, it may cause additional damage to stored products by spreading and promoting fungal contamination. Insect-microbiota interactions play an important role in insect biology, influencing the insect's development, physiology, nutrition, survival, immunity, or even vector competence (Malassigné *et al*., 2021).

In 2021, individuals of corn weevil were observed in corn seed samples from seed lots that usually showed high fungal contamination. When the seeds were placed in a culture medium, a yeast was found and no fungal contaminants were isolated. We hypothesized that the unknown yeast species carried by the corn weevils is bioactive against mycotoxigenic fungal seed contaminants. This study aims to 1) identify the yeast species isolated from the corn weevils using a combined conventional and DNA-based approach and 2) assess the antifungal activity of this yeast against three common mycotoxigenic fungi *F. verticillioides, A. flavus,* and *A. niger* using spread plate technique and dual culture assays.

Materials and Methods

Sample collection, yeast isolation and mycotoxigenic fungi source

Individuals of corn weevil were collected in March 2021 in Laguna, Philippines, from brown paper bags containing corn seeds. The weevils were kept in glass flasks with a mesh screen lid. For the experiment, weevils were directly plated onto Petri plates (10 individuals/plate X 3 plates) containing potato dextrose agar (PDA) medium. The plates were incubated at room temperature (28-30°C) for seven days. The yeast was isolated and purified in new PDA medium.

The three mycotoxigenic fungi used in this study, *A. flavus, A. niger,* and *F. verticillioides,* were obtained from the fungal repository of the Plant Pathology Laboratory, Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines Los Baños. These fungi were previously isolated from stored corn seeds and were already identified using molecular assays. The fungi were also found to contain the aflatoxin, ochratoxin, and fumonisin biosynthesis genes and metabolites, determined using a polymerase chain reaction (PCR) assay and ELISA kit, respectively.

Yeast identification

Identification of the yeast species was done through a combined morpho-cultural and molecular assays. Morphocultural characterization was done in yeast extract peptone glucose agar (YEPG) (10 g yeast extract, 20 g peptone, 10 g glucose, 15 g agar). The insect genomic DNA was extracted using the CTAB method (Doyle and Doyle, 1987). The sequences of the D1/D2 domain using the LR0R (ACCCGCTGAACTTAAGC) and LR5 (TCCTGAGGGAAACTTCG) (Vilgalys, 1988) primer pairs were used to amplify the LSU rRNA gene region of the yeast in a PCR assay. The 25 µL PCR mix contained 1x PCR buffer (Invitrogen), two mM MgCl2 (Invitrogen), 0.2 mM dNTP mix (Invitrogen), 0.2 µM of each primer, 1 U of *Taq* polymerase (Invitrogen), one µL DNA template and DEPC-treated water (Invitrogen). PCR reactions were performed using MyCycler™ Thermal Cycler System (Bio-Rad Laboratories) with an initial denaturation for 5 min at 95°C, 35 cycles of 30-sec denaturation at 95°C, 30-sec annealing at 55°C, and 1 min extension at 72°C, followed by a final extension for 10 min at 72°C for LSU rRNA gene and an initial denaturation for 5 min at 94°C, 35 cycles of 30-sec denaturation at 94°C, 30-sec annealing at 55°C, and 30-sec extension at 72°C.

The amplified products were visualized using the Molecular Imager GelDoc™ XR+ with Image Lab software (Bio-Rad Laboratories), and amplified PCR products were sent to 1st Base (Malaysia) for DNA sequencing.

Weevil Identification

A PCR assay, using the same conditions as mentioned above, was also performed to validate the identity of the corn weevil. Primer pairs LCO1490 (GGTCAACAAATCATAAAGATATTGG) and HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA) (Folmer *et al*., 1994) of the COI gene were used in the PCR assay.

DNA Sequence Analysis

Sequence assembly was performed using the Geneious program. The obtained consensus sequences were compared pairwise using a BLASTN search (NCBI GenBank). Sequences were then aligned with the sequences of related species retrieved from GenBank using multiple alignments. A phylogenetic tree was constructed using the maximum likelihood method of MEGA X software, and confidence levels of the clades were estimated from bootstrap analysis (1,000 replicates).

***In vitro* screening of yeast antagonistic assay**

Hyphopichia burtonii was tested on each fungal isolate to determine the yeast's effect on fungal growth and spore production in a spread plate technique following the method of Souza *et al.* (2017). The yeast was grown in culture media and a concentration of 10^7 cells mL^{-1} was obtained. For the fungal isolates, suspension of spores was obtained at 10^6 spores mL^{-1}. Aliquots of 100 µL of the yeast suspension were spread using a Drigalsky handle on Petri dishes containing PDA medium. Then, aliquots of 10 µL of each fungal spore suspension were placed in the center of the Petri dishes. This was performed in triplicates and the Petri dishes were incubated at 28°C for seven days. Positive control for the growth of each fungal isolate was conducted by inoculating the spores without inoculating the yeast isolates. The colony diameter of each fungus was measured to evaluate the mycelial vegetative growth, and the percentage of growth inhibition was obtained, considering that the positive control was 100% of the diameter. The same treatments for growth inhibition were conducted to analyze spore production. After seven days, the spores were counted by placing three agar blocks per replicate in 10 ml of sterile distilled water. The percentage of inhibition of spore production was obtained considering the spore concentration in the positive control as 100% for each fungal isolate.

A dual culture assay (Moradi *et al*., 2020) was also performed by placing 50 µl of an actively growing suspension of *H. burtonii* (10^8 cells mL^{-1}) and streaking 3.5 cm away from the center of the YEPG plates containing 10 g yeast extract, 20 g peptone, 10 g glucose, and 15 agar and subsequently incubated at 25°C for 24 h. Post-incubation, 10 µL each of the three mycotoxigenic fungal spore suspensions (10^6 spores mL^{-1}) was placed in the center of each Petri dish and incubated at 28°C in the dark. The inhibition of radial fungal growth was recorded for up to 7 days. Fungal growth with no yeast inoculum was used as a control. The ability of strains to inhibit fungal growth was calculated with the following equation: $I = C\text{-}T/C \times 100$, where I is the inhibition of mycelial growth (%), C is the growth of the fungal pathogen in control Petri dishes, and T is the growth in the interaction assays. The percentage of inhibition of spore production was also conducted.

Effect of heating (volatile and non-volatile compounds) on the antimicrobial activity of *H. burtonii*

To assess the effects of volatile compounds on the mycelial growth of fungi, the *H. burtonii* strain was streaked out on YEPG plates and incubated for two days at 28°C (Moradi *et al.,* 2020). Ten µL each of the three mycotoxigenic fungal spore suspensions (10^6 spores mL^{-1}) of 7-day-old fungi were placed in the center of the plate and upside down on a Petri dish containing the 48-hour inoculated *H. burtonii* strain. The plates were

then sealed with paraffin film (Parafilm, Sigma-Aldrich, Germany) and incubated for seven days at 27°C in the dark. The fungi's colony diameter (mm) was measured after seven days. The YEPG plates with no yeast isolate applied were used as a control.

In the non-volatile compound assay, *H. burtonii* strain was cultured in individual flasks containing 75 ml of sterilized potato-dextrose broth (PDB). It was placed on a rotary shaker (150 rpm) at room temperature to promote the growth of yeast strain. After four days, the suspensions were passed through No. 1 filter paper (Whatman, Sigma-Aldrich, Germany) and autoclaved. The sterilized suspension was mixed with YEPG at three ratios (5%, 15%, and 25%) and poured into Petri dishes. Fungal suspensions were inoculated on the center of the plates at 10 µL (10^6 spores mL^{-1}). The mycelial growth of the fungi was monitored and recorded at seven days.

Statistical Analysis

A paired sample (Independent) T-test and ANOVA test were performed using Statistical Tool for Agricultural Research (STAR Nebula) with a 95% confidence level.

Results

Yeast incidence and identity

The yeast grew from all corn weevils (100% incidence) plated in the PDA medium after seven days (Fig. 1A). No other microbes were observed growing from the corn weevil samples. Morphocultural characterization showed white, powdery, flat, filamentous colonies with yeast-like cells which are ellipsoidal or pyriform in shape at about 2.5-5.0 x 1.5-2.5 µm with thin, smooth walls with septate hyphae that are 4-6 µm broad, and dichotomously branched (Fig. 1B and 1C). Analysis of the D1/D2 domain of the LSU rRNA gene amplified a PCR product of 860 bp (Fig. 2B) and NCBI BLAST results showed 100% similarity to *Hyphopichia burtonii* (CP024760) (Table 1). The phylogenetic tree was generated based on the sequences of the D1/D2 domain of the LSU rRNA gene also revealed that the strain formed a cluster with known *H. burtonii* (Fig. 3).

Weevil identity

PCR product of the weevil's DNA analyzed using COI gene showed 700 bp at 1.5% agarose gel electrophoresis (Fig. 2A). The DNA sequences of the COI gene of the individuals revealed 99.14% similarity to *Sitophilus zeamais* (NC030764) (Table 2).

Yeast's antifungal activity

Four *in vitro* assays were conducted to test antifungal activity of *H. burtonii* against three mycotoxigenic fungi. In the spread plate assay, *H. burtonii* significantly inhibited the colony growth of three fungi with a 50-69% reduction on spread plate assay (Fig.

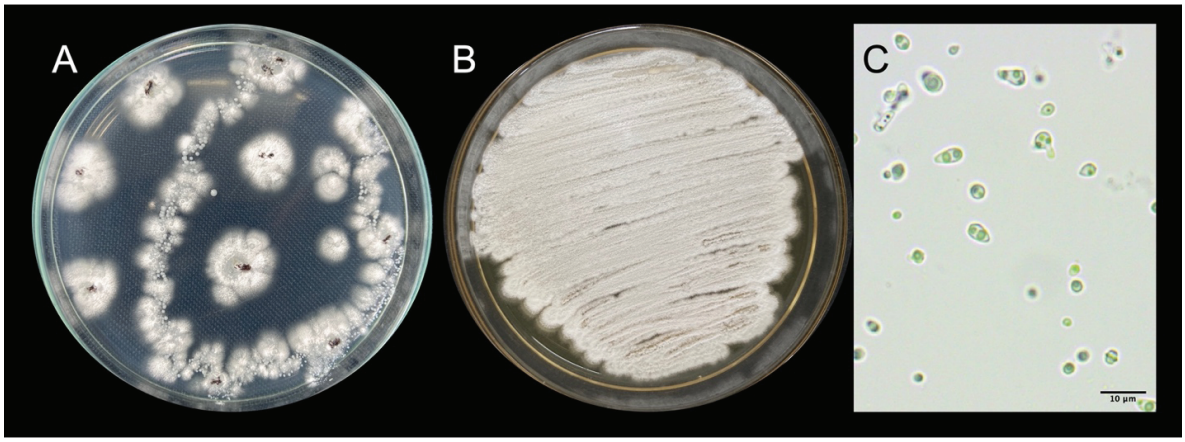

Figure 1. Isolation of mycoflora in corn weevil by direct plating on PDA: (A) pure culture of *Hyphopichia burtonii* grown in yeast extract peptone glucose agar (YEPG) at 3 days incubation (B) and yeast cells at 1000x magnification (bar=10 µm) (C).

4A and Fig. 5). Spore production was also inhibited at 57-96% where highest inhibition was recorded with *A. flavus* at 96%, followed by *A. niger* at 87.85% and *F. verticillioides* at 57.63% (Table 3). In the dual culture assay, the isolate significantly inhibited the growth of *F. verticillioides* and *A. niger* at 13.68% and 6.31% respectively. However, no significant inhibition was observed on *A. flavus* (Fig. 4B and Fig. 6). On fungal spore production, all fungi were significantly inhibited (29-40%) (Table 4). *H. burtonii* inhibited the mycelial growth of the three fungi which is correlated with the inhibition of spore production.

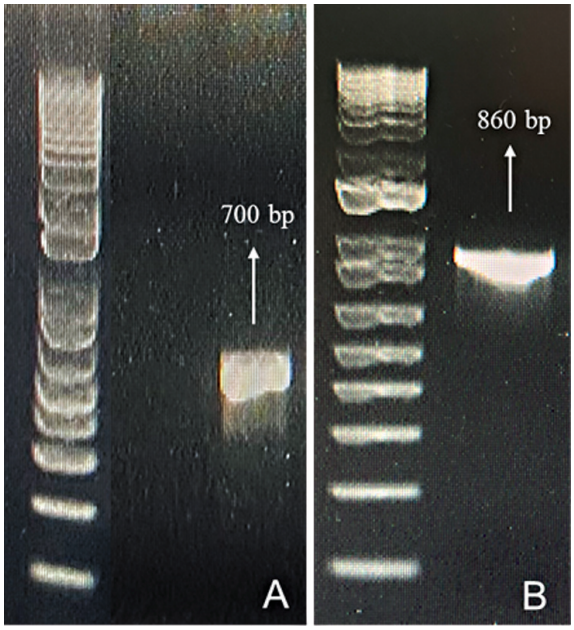

Figure 2. Agarose gel electrophoresis (1.5%) of PCR product of *Sitophilus zeamais* at 700 bp using COI gene (A) and *Hyphopichia burtonii* at 860 bp using LSU rRNA gene (B).

The test for the presence of volatile compounds of *H. burtonii* also showed a reduction in mycelial growth in *F. verticillioides* and *A. niger* with 17.41 and 58.89% inhibition, respectively, while *A. flavus* growth was not significantly affected (Fig. 7A). For the non-volatile compound test, higher yeast concentration amended in YEPG (15% and 25%) was efficient in inhibiting the growth of *F. verticillioides* (25-30% inhibition) and *A. flavus* (7.41-14.44%) but did not affect *A. niger* growth (Fig. 7B). At 5% yeast concentration, no inhibition was observed in *F. verticillioides* and *A. flavus*. However, significant

Table 1. Sequences retrieved from GenBank according to the closest BLAST results of the D1/D2 domain of the LSU rRNA gene.

Isolate	Closest sequence match	Source/Host	Country	Coverage	Similarity
MB Corn Weevil Yeast Isolate 001	CP024760.1 *Hyphopichia burtonii*	nuruk	South Korea	100	100
	KY107882.1 *Hyphopichia burtonii*	inse	NI	100	100
	HF952839.2 *Hyphopichia burtonii*	Farm silage	Netherlands	100	100
	MH867400.1 *Hyphopichia burtonii*	NI	Netherlands	99	100
	NG_054819.1 *Hyphopichia burtonii*	NI	NI	96	100
	CP024761.1 *Hyphopichia burtonii*	nuruk	South Korea	88	100
	KY106445.1 *Hyphopichia fennica*	NI	Finland	95	97.09
	KY107885.1 *Hyphopichia burtonii*	Homo sapiens	NI	82	100
	JQ733412.1 *Pichia* sp.	Halophila ovalis	China	93	96.15
	CP024757.1 *Hyphopichia pseudoburtonii*	nuruk	South Korea	100	90.41

Abbreviations: (NI) No information.

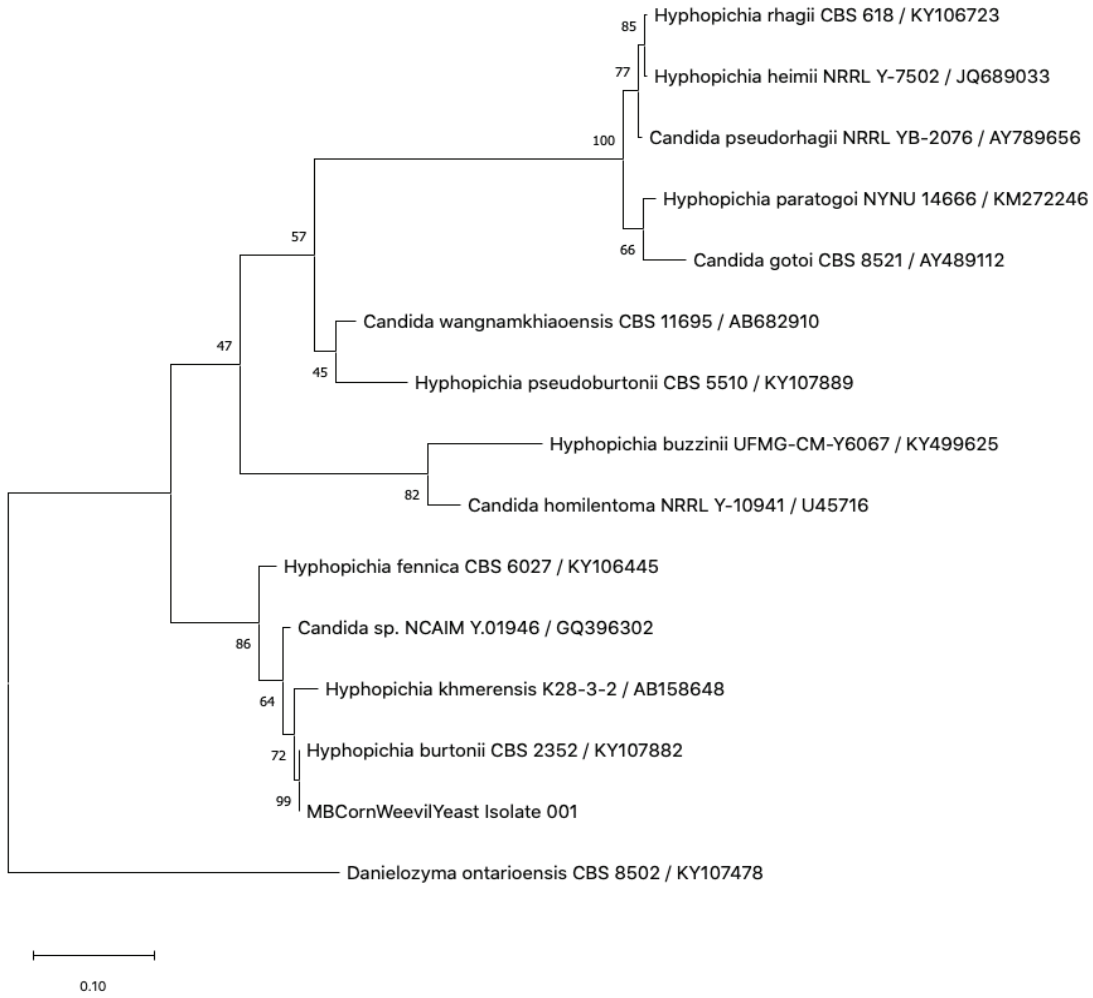

Figure 3. Phylogenetic tree based on the sequences of the D1/D2 domain of the LSU rRNA gene, showing positions of *Hyphopichia burtonii* with respect to closely related species. The phylogenetic tree was constructed from evolutionary distance data using maximum likelihood method. The numbers at nodes indicate the percentages of bootstrap sampling, derived from 1,000 samples. *Danielozyma ontarioensis* was the outgroup species in the analysis.

inhibition was observed in *A. niger* at the lowest yeast concentration.

Discussion

The corn weevil, *S. zeamais*, is known to be a vector of fungi, some of which produce toxins (aflatoxin and fumonisin). In this study, a yeast was isolated from individuals of corn weevil using combined morpho-cultural and molecular assays, and was identified as *H. burtonii*. To our knowledge, this is the first scientific report of *H. burtonii* isolated from a storage-product insect pest. As there were no fungi growing on weevils where the yeast was isolated, it was hypothesized that the yeast inhibited the activity of mycotoxigenic fungi. Yeast has been regarded as potential biocontrol agent against toxigenic fungi in stored grains (Petersson and Schnürer, 1998; Petersson *et al*., 1998; Masoud and Kaltoft, 2006). In the current study, we confirm that the *H. burtonii* isolated from corn weevil had inhibitory activity on the growth and spore production of three mycotogenic fungi, namely *A. flavus, A. niger,* and *F. verticillioides*.

The morphology and colony features of the *H. burtonii* isolated strain in this study corroborate with the description of Arx and

Table 2. Sequences retrieved from GenBank according to the closest BLAST results of cytochrome oxidase subunit I (COI) gene.

Isolate	Closest sequence match	Source/Host	Country	Coverage	Similarity
MBCornWeevil	NC_030764.1 *Sitophilus zeamais*	NI	NI	99	99.14
	AY131100.1 *Sitophilus zeamais*	NI	NI	99	99.14
	MT294139.1 *Sitophilus zeamais*	Sorghum grains	China	99	98.99
	MN905575.1 *Sitophilus zeamais*	NI	China	99	98.99
	KU757289.1 *Sitophilus zeamais*	NI	China	99	98.99
	MK649856.1 *Sitophilus zeamais*	NI	NI	94	100
	OQ533509.1 *Sitophilus zeamais*	NI	Pakistan	94	99.85
	KY912951.1 *Sitophilus zeamais*	NI	China	94	99.85
	KY912950.1 *Sitophilus zeamais*	NI	China	94	99.85
	KM459446.1 *Sitophilus zeamais*	NI	India	94	99.85

Abbreviations: (NI) No information.

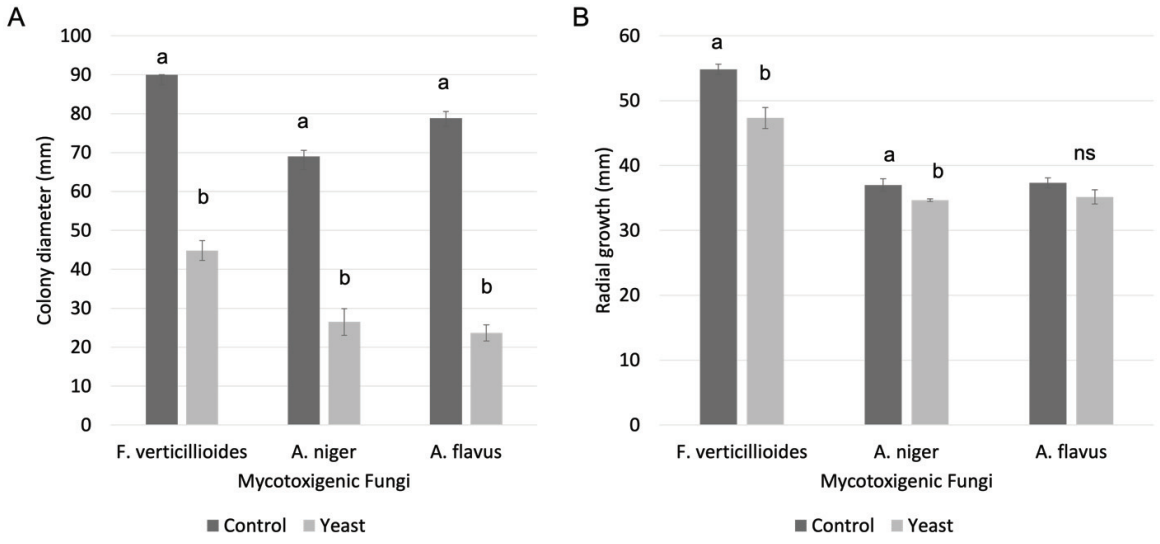

Figure 4. Colony growth of three mycotoxigenic fungi using spread plate (A) and dual culture test (B) of *Hyphopichia burtonii* at 7 days incubation. Bars, within a figure, with different letter indicate means were significant at $p<0.05$ based on T-test analysis. Values represent the mean of the two trials performed.

Vab Der Walt (1976) and Pitt and Hocking (2009). This species was formerly known as *Pichia burtonii*, a wide-spread yeast causing food and beverage spoilage. It is also called «chalk molds» because it causes defects on partially baked bakery products, cured

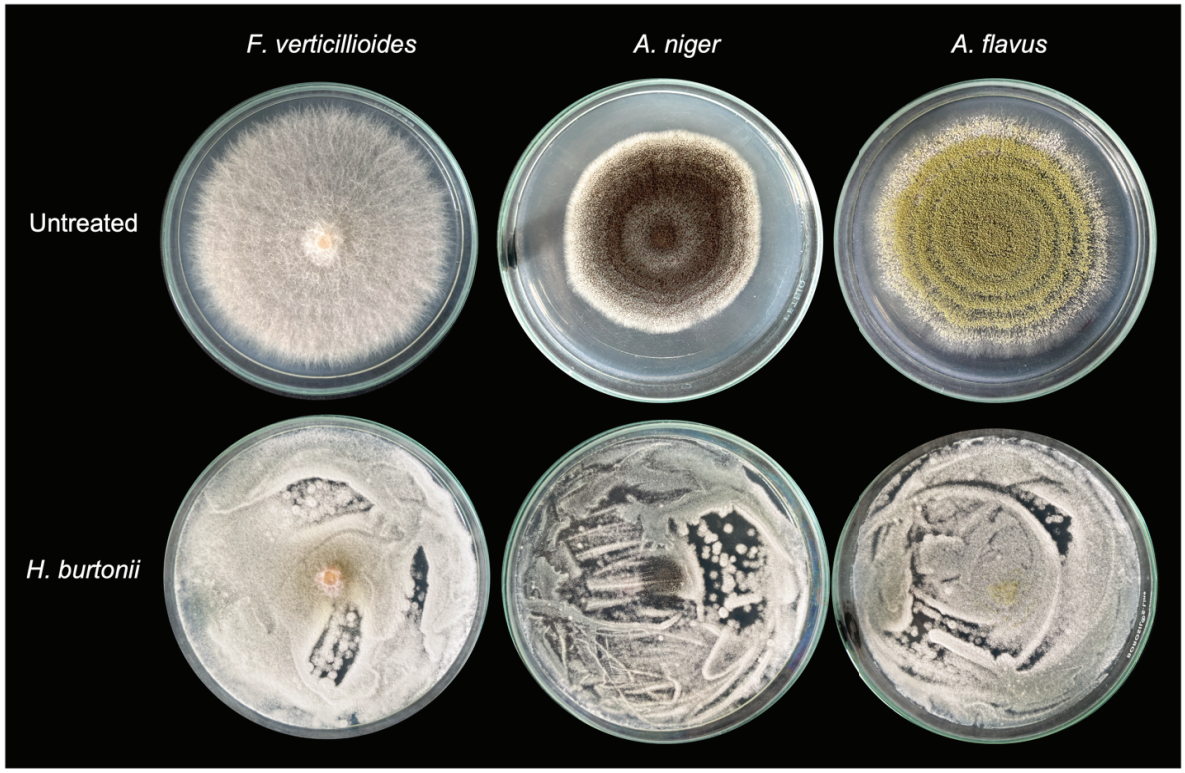

Figure 5. Spread plate assay of the antagonistic activity of *Hyphopichia burtonii* against three mycotoxigenic fungi: *Fusarium verticillioides, Aspergillus niger, Aspergillus flavus* (L-R).

Table 3. Colony growth and spore inhibition of three mycotoxigenic fungi to *Hyphopichia burtonii* strain using spread plate assay.

Treatment	Colony diameter (mm)			Spores/ml		
	F. verticillioides	*A. niger*	*A. flavus*	*F. verticillioides*	*A. niger*	*A. flavus*
Control	90.00a	69.00a	78.83a	$1.97x10^5$a	$4.06x10^6$a	$6.63x10^6$a
H. burtonii	44.83b	26.50b	23.67b	$8.33x10^4$b	$4.93x10^5$b	$2.65x10^5$b
Inhibition(%)	50.19	61.59	69.98	57.63	87.85	96.00

meat, and cookies (Lee and Fujio, 1999; Simoncini *et al*., 2007). The species has been reported in a wide variety of substrates, particularly high starch substrates such as corn, wheat, and rice, and from insects and water from fish ponds (Kurtzman, 2011). *Hyphopichia burtonii* has been isolated from grass insects in Thailand (Limtong *et al*., 2012), coffee berry borer (*Hypothenemus hampei*) from a coffee tree in Brazil (Moreira, 2012), wild edible crickets (*Gryllus bimaculatus)* in Kenya (Gatheru, 2019), red flour beetle (*Tribolium castaneum)* in rice in Korea (Yun *et al*., 2018), guts of insect larvae and decayed woods from Henan Province, Central China (Ren *et al*., 2015), and insect frasses (Kurtzman, 2011). In addition, *H. burtonii* has also been reported in rotting woods, the leaf surface of a mango tree, and a freshwater lake in Brazil (Ribeiro *et al*., 2017), spoiled foodstuffs, caterpillars, silage, pollen, fish feeds, and fishponds (Pinheiro *et al*., 2018; Barnett *et al*., 2000). The current study, to our knowledge, is the first scientific report of *H. burtonii* isolate from corn weevil (*S. zeamais*).

Moreira (2012) reported that *H. burtonii*

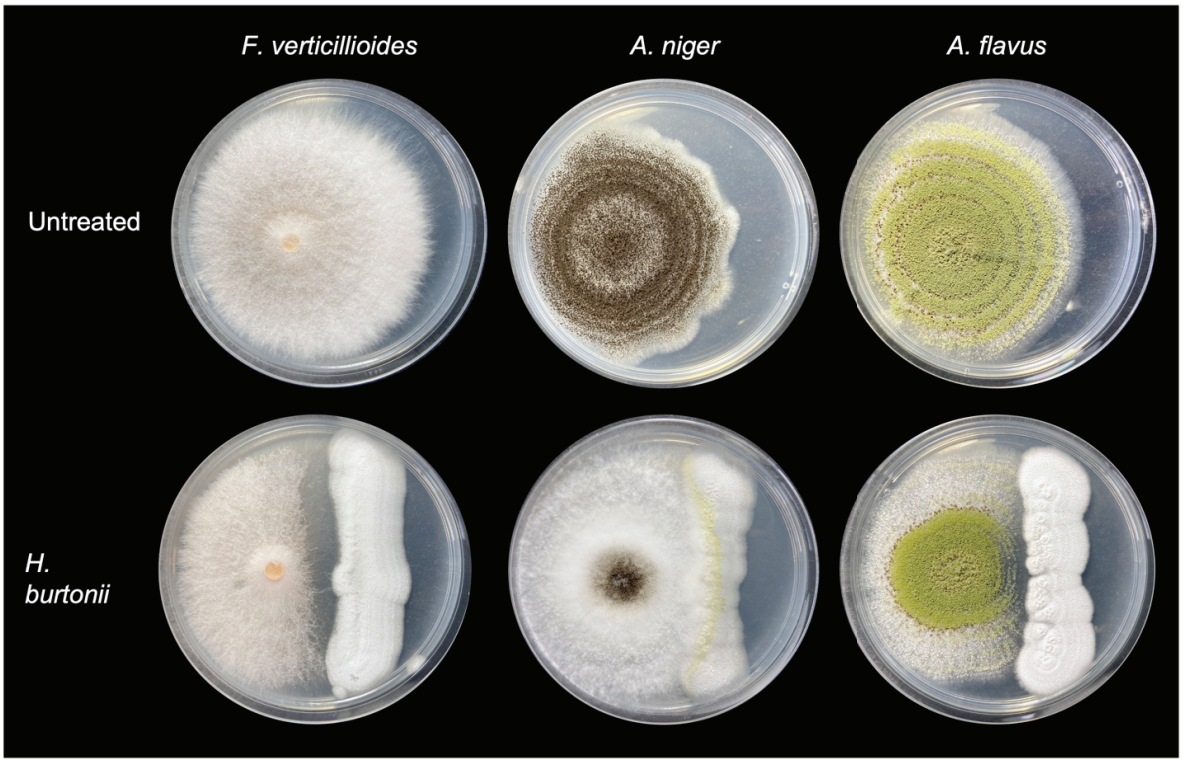

Figure 6. Dual culture assay of the antagonistic activity of *Hyphopichia burtonii* against three mycotoxigenic fungi: *Fusarium verticillioides, Aspergillus niger, Aspergillus flavus* (L-R).

Table 4. Colony growth and spore inhibition of three mycotoxigenic fungi to *Hyphopichia burtonii* strain using dual culture assay.

Treatment	Radial growth (mm)			Spores/ml		
	F. verticillioides	*A. niger*	*A. flavus*	*F. verticillioides*	*A. niger*	*A. flavus*
Control	54.83a	37.00a	37.33ns	1.7 x10^5a	6.2 x10^6a	4.2 x10^6a
H. burtonii	47.33b	34.67b	35.17	1.0 x10^5b	3.5 x10^6b	3.0 x10^6a
Inhibition(%)	13.68	6.31	5.80	40.00	43.39	28.58

parasitizes the coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae). In the current study, we did not observe *H. burtonii* parasitizing the corn weevil. Nevertheless, further research is necessary to determine the associations of *H. burtonii* with the corn weevil, aside from the latter being a vector of the yeast. *Hyphopichia burtonii* has also been associated with cutaneous infection in Barbastelle bats (Simpson *et al*., 2013). In 2021, a strain of *H. burtonii* was reported, for the first time, to be associated with peritonitis in humans on peritoneal dialysis, which may require specific media and other detection techniques (Chamroensakchai *et al.,* 2021). The yeast strain associated with the corn weevil in our study was easily cultured in PDA medium within seven days. Further research is needed to compare the biology and pathology of *H. burtonii* isolates from various sources as these may differ based on their host (pathogenic or symbiotic) and host specificity is also possible. A genomics approach may also shed light as to whether the *H. burtonii* isolates from various substrates and samples (plants, insects, and

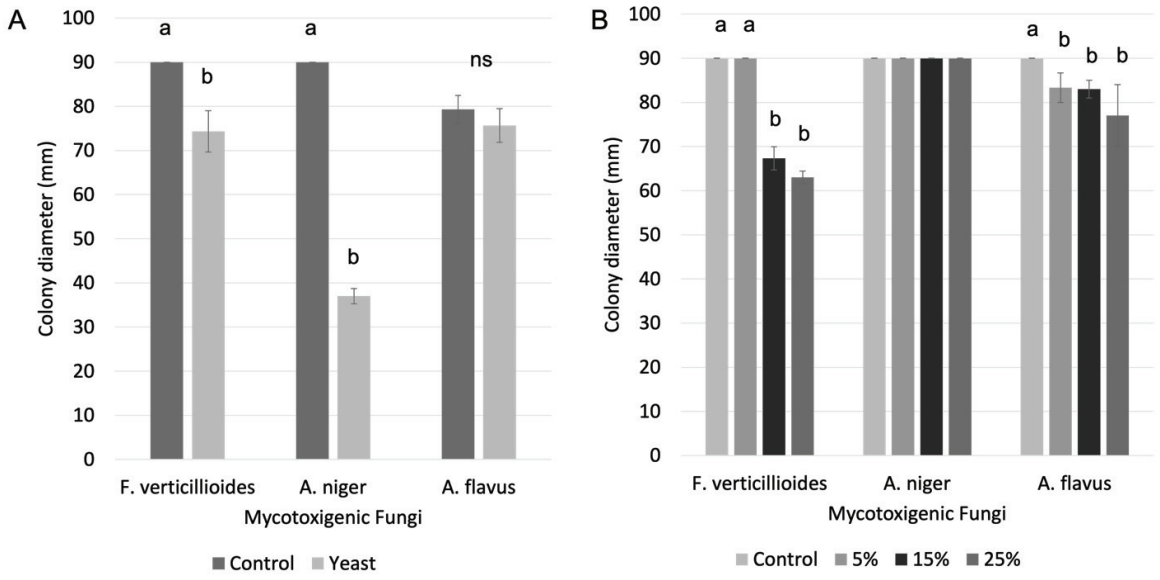

Figure 7. Colony diameter (mm) of three mycotoxigenic fungi on test for volatile (A) and non-volatile compounds (B) of *Hyphopichia burtonii* at 7 days incubation. Bars, within a figure, with different letter indicate means were significant at $p<0.05$ based on T-test analysis and ANOVA test.

animals) are the same or different strains. Care must be considered when working on stored grains and other products (bread, beverages, and other substrates) where *H. burtonii* has been previously reported.

The role of *H. burtonii* in insects and other hosts is not yet fully understood. Studies have indicated that it might play important roles in their hosts by detoxification of food materials and help on the supply of essential nutrients (Ren *et al*., 2015) or parasitizing insects and other hosts (Moreira, 2012; Simpson *et al*., 2013; Chamroensakchai *et al*., 2021). In the present study, we showed the antifungal activity of *H. burtonii* isolated from corn weevil to the growth and spore production of three mycotoxigenic fungi *in vitro*. The results showed that the method of strain inoculation (yeasts and fungi) differed in the level of its inhibition effect on mycelial growth and spore production. *Hyphopichia burtonii* highly-suppressed the mycelial growth and spore production of the three fungi when spreading the yeast isolate in the agar prior to the inoculation of the fungi. The results corroborate with those of Souza *et al*. (2010), where the production of toxigenic fungal spores was inhibited by 100% by wild yeasts.

In contrast, dual culture significantly inhibited mycelial growth and spore production of the three fungi but at a lower rate compared with the spread-plated yeast. A difference in the degree of inhibition by the yeast isolate was also observed among the three mycotoxigenic fungi: the lowest inhibition was obtained against *A. flavus*, followed by *A. niger*, then *F. verticillioides*. In the case of *A. flavus,* the yeast inhibited sporulation but did not interfere with mycelial growth. Unlike the spread plate assay, the dual test allowed the growth of the yeast and the fungi on each side, thus, giving time for the fungi to grow before interacting with the yeast isolate. It could be that the *Aspergillus* species spreads faster than *F. verticillioides* and explains the low inhibition effects on these two species. Similarly, Ramos *et al*. (2010) explained yeast isolates' more significant inhibitory effect on sporulation than on mycelial development when they inoculated the yeast isolates concomitantly with the inoculation of filamentous *Aspergillus* fungi, namely *A. carbonarius* and *A. ochraceus*, 4 cm apart, allowing both isolates to develop before the direct contact of the yeast colony with the fungi. Moreover, Ramos *et al*. (2010) and Souza *et al*. (2017) re-

ported that the concentration of the yeast inoculum and the degree of inhibition were directly proportional: the higher the inoculum concentration of the yeast species, the greater the inhibitory effect on sporulation.

The volatile and non-volatile tests in *H. burtonii,* indicate that the yeast may contain volatile and non-volatile compounds with different effect on the fungi at different yeast concentrations since the volatile compounds significantly inhibited *A. niger,* while non-volatile compounds at concentration 15% inhibited mycelial growth of *F. verticillioides* (25% inhibition). and at concentration 5%, of *A. flavus* (7% inhibition).

Overall, the different inhibition effect of *H. burtonii* on the three mycotoxigenic fungi mycelium growth and spore production in dual culture, volatile compound, and non-volatile compound tests, can be related to different mechanisms of competitive interactions between the yeast strain and the fungi. A high yeast population may likely restrict the availability of nutrients and sites for colonization, which are essential for the germination of spores (Björnberg and Schnürer, 1993). Antagonistic yeasts possess several mechanisms of action, including competition for nutrients and space, production of cell wall degrading enzymes, volatile organic compounds, non-volatile compounds, and direct mycoparasitism (Freimoser *et al*., 2019). Volatile compounds are known to act directly against the pathogens (direct antibiosis) by destroying the cell wall or indirectly inducing systemic resistance to the plant (Chen *et al*., 2008; Zheng *et al*., 2013). Identification of these compounds may lead to the their use as chemical agents against mycotoxigenic fungi.

Conclusion

The yeast *H. burtonii* which was isolated from the storage pest corn weevil (*S. zeamais*) for the first time was antagonistic to three mycotoxigenic fungi (*A. flavus, A. niger,* and *F. verticillioides)* of corn by inhibiting fungal growth and spore production. Thus, the research contributes with knowledge for the potential utilization of *H. burtonii* against mycotoxigenic fungi, and the reduction of mycotoxins in stored products.

Declarations

Author contributions

Conceptualization, design and supervision, MAB and AKB; conduction of experiments, design and generation of figures , analysis of data and writing—original draft preparation, MNS; interpretation of results, MNS and MAB; writing—review, editing and final version of the manuscript, MAB and AKB. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

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Πρώτη καταγραφή του *Hyphopichia burtonii* που απομονώθηκε από το έντομο αποθηκών *Sitophilus zeamais* και η βιοδραστηριότητά του έναντι μυκοτοξινογόνων μυκήτων

M.N.P. Seco, A.K.M. Beltran και M.A.O. Balendres

Περίληψη Ο βρούχος του καλαμποκιού (*Sitophilus zeamais*) είναι ένα από τα πιο καταστροφικά έντομα των σπόρων του αραβοσίτου κατά την αποθήκευση. Το έντομο δύναται να είναι φορέας μυκοτοξινογόνων μυκήτων ή ζυμομυκήτων που επιμολύνουν τους σπόρους. Σε αυτή τη μελέτη, ένα άγνωστο είδος ζυμομύκητα απομονώθηκε από άτομα του *S. zeamais* που εντοπίστηκαν σε αποθηκευμένους σπόρους αραβοσίτου. Υποθέσαμε ότι ο συγκεκριμένος ζυμομύκητας είχε αντιμυκητιακή δράση αναστέλλοντας την ανάπτυξη μυκοτοξινογόνων μυκήτων στους σπόρους του αραβοσίτου. Το είδος του ζυμομύκητα ταυτοποιήθηκε ως *Hyphopichia burtonii*, με τη συνδυασμένη εφαρμογή μορφολογικών και μοριακών μεθόδων, και αξιολογήθηκε *in vitro* (επίστρωση σε τριβλίο PDA και διπλή καλλιέργεια) η πιθανή ανασταλτική δράση του στην ανάπτυξη τριών γνωστών μυκοτοξινογόνων μυκήτων,

Fusarium verticillioides, Aspergillus niger και *A. flavus*. Ο έλεγχος της ανταγωνιστικής δράσης της απομόνωσης του ζυμομύκητα έδειξε 50 – 69% και 5,8-13,7% αναστολή ανάπτυξης της αποικίας των μυκήτων, όταν χρησιμοποιήθηκαν οι μέθοδοι της επίστρωσης σε τριβλίο και της διπλής καλλιέργειας, αντίστοιχα. Επίσης επηρεάστηκε η παραγωγή σπορίων από τους τρεις μύκητες σε ποσοστό 57 – 96% και 29 – 40%, στη μέθοδο της επίστρωσης σε τριβλίο και της διπλής καλλιέργειας, αντίστοιχα. Επιπλέον, τα πτητικά και τα μη πτητικά κλάσματα προκάλεσαν μείωση στην ανάπτυξη των μυκηλίων. Παρατηρήθηκαν διαφορετικές αποκρίσεις μεταξύ των μυκοτοξινογόνων μυκήτων. Περαιτέρω έρευνα θα είχε ενδιαφέρον για την πιθανή χρήση του ανταγωνιστικού ζυμομύκητα για τη μείωση της ανάπτυξης του μυκηλίου και της σποριοποίησης των μυκοτοξινογόνων μυκήτων και τον πιθανό μετριασμό της επιμόλυνσης σπόρων αραβοσίτου με μυκοτοξίνες. Από όσο γνωρίζουμε, αυτή είναι η πρώτη καταγραφή απομόνωσης του ζυμομύκητα *H. burtonii* από έντομο και ειδικότερα από το *S. zeamais*.